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International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : A61K 7/48, 33/24, 31/28

A1

(11) International Publication Number: WO 90/12563

(43) International Publication Date: 1 November 1990 (01.11.90)

(21) International Application Number: PCT/US90/02175

(22) International Filing Date: 20 April 1990 (20.04.90)

(30) Priority data:
342,993 24 April 1989 (24.04.89) US

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(81) Designated States: AT, AT (European patent), AU, BB, BE (European patent), BF (OAPI patent), BG, BJ (OAPI patent), BR, CA, CF (OAPI patent), CG (OAPI patent), + CH, CH (European patent), CM (OAPI patent), DE, + DE (European patent), DK, DK (European patent), ES, ES (European patent), FI, FR (European patent), GA (OAPI patent), GB, GB (European patent), HU, IT (European patent), JP, KP, KR, LK, LU, LU (European patent), MC, MG, ML (OAPI patent), MR (OAPI patent), MW, NL, NL (European patent), NO, RO, SD, SE, SE (European patent), SN (OAPI patent), SU, TD (OAPI patent), TG (OAPI patent).

Published
With international search report.
Before the expiration of the time lim[illegible] for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: COMPOSITIONS AND PROCESSES FOR IMPROVING THE COSMETIC APPEARANCE, GROWTHS OR HEALING CHARACTERISTICS OF TISSUE

(57) Abstract

The most preferred aspect of the present invention comprises a method of alleviating skin wrinkles and other effects of aging on the skin using vanadium formulations or compositions. The method includes repeatedly applying vanadium compositions over a period of time.

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Compositions and processes for improving the cosmetic appearance, growth or healing characteristics of tissue.

Aging and sun exposure may result in folds, lines, wrinkles and loss of skin elasticity. Such results are regarded as unattractive in many cultures. At present, retinoids are the only agents convincingly shown to alleviate wrinkles and other effects of aging on the skin, but they may be toxic and irritating.

The element vanadium is a gray or white, maleable, ductile and polyvalent metal. Vanadium can be found in the environment and occurs in various concentrations in soil, water, air, plants, and animal tissues.

Although vanadium has been proposed to be an essential nutritional element, this has not been unequivocally established. Vanadium-containing compounds have been used in the form of water-soluble vanadium salts on skin, but only as an antiperspirant (United States Patent No. 4,490,354). Such use was in far higher quantities, as discussed subsequently, than are necessary to provide the advantages of the invention described herein. The only other expressed medical, nutritive or cosmetic use of vanadium is as a listed ingredient in some multiple vitamin-mineral preparations. However, the role of vanadium in biochemical processes remains poorly understood.

The present invention relates to the use of solubilized vanadium in methods and compositions useful and particularly effective for treating mammalian tissues and mammals per se for internal healing and, especially with respect to alleviating dermal conditions such as skin wrinkles, reducing actinic keratoses, and healing damaged tissue. In light of the existing knowledge, the novel effects of vanadium in the instant invention could not have been predicted.

Vanadate ion alone, or in combination with other enhancing adjuvants, in concentrations and doses carefully selected in view of a specific deleterious tissue condition without or within a mammalian body, will at least partially alleviate such condition. The vanadate ion concept of this invention is described in particularity as an especially preferred embodiment of this invention for treating wrinkles and healing skin injuries. This concept, however, is not so limited, the generic concept being the use of vanadate ion in controlled preselected concentrations to alleviate a wide variety of mammalian deleterious conditions and to enhance mammalian tissue structure, even in a culture environment extrinsic to a body.

Figure 1A is a frontal view of a human subject from Example 1 before treatment.

Figure 1B is a frontal view of the subject from Example 1 after 40 days of application of a vanadate composition (described below) twice daily to the right side of his face and application of the vehicle alone (placebo) used in the vanadate composition twice daily to the left side. Both sides of the neck were treated twice daily with the same vanadate compositi n.

Figure 1C is a frontal view of the subject from Example 1 after 48 days of twice daily application of the vanadate composition to the right side and placebo application to the left side. Both sides of the neck were treated twice daily with the vanadate composition.

Figure 1D is frontal view of the subject from Example 1 after 58 days of twice daily application of the vanadate composition to the right side and placebo application to the left side. Both sides of the neck were treated twice daily with the vanadate composition.

Figure 1E is a left perspective view of the subject from Example 1 after 48 days of twice daily treatment with placebo. Both sides of the neck were treated twice daily with the vanadate composition.

Figure 1F is a right perspective view of the subject from Example 1 after 48 days of twice daily treatment with a vanadate composition. Both sides of the neck were treated twice daily with the vanadate composition.

Figure 1G is a left perspective view of the subject from Example 1 after 58 days of twice daily treatment with placebo. Both sides of the neck were treated twice daily with the vanadate composition.

Figure 1H is a right perspective view of the subject from Example 1 after 58 days of twice daily treatment with a vanadate composition. Both sides of the neck were treated twice daily with the vanadate composition.

Figure 1I is a left perspective view of the subject from Example 1 after 86 days of twice daily treatment with a vanadate composition. B th sides of the neck were treated twice daily with a vanadate composition.

Figure 1J is a right perspective view of the subject from Example 1 after 144 days of twice daily treatment with a vanadate composition. Both sides of the neck were treated twice daily with the vanadate composition.

Figure 2A is a frontal view of the 47-year old subject from Example 2 before treatment.

Figure 2B is a frontal view of the subject from Example 2 after 16 days of application of a vanadate composition (described below) twice daily to the left side of the face and application of the vehicle used in the same vanadate composition, herein called 'placebo,' twice daily to the right side of the face.

Figure 3A is a dorsal view of a subject's hands from Example 3 forty-eight hours after actinic keratoses were frozen with liquid nitrogen. Twenty-four hours after liquid nitrogen application, the hands were treated on a twice a day basis. The right hand was treated with vanadate, and the left hand was treated in the same manner with the vehicle used for the vanadate mixture (placebo).

Figure 3B is a dorsal view of the subject's hands from Example 3 after four days of treatment with vanadate (right hand) and with placebo (left hand).

Figure 3C is a dorsal view of the subject's hands from Example 3 after fourteen days of treatment with vanadate (right hand) and with placebo (left hand).

A basic conceptual feature of this invention is as follows. Before conversion to vanadyl ions, vanadate ions, the form of vanadium present in body fluids of warm bl oded animals, including humans, resemble phosphate

ions. Such vanadate ions, therefore, have potentially many biochemical sites of action. Phosphate ions are known to participate extensively in biochemical reactions, and vanadate ions, as analogues of phosphate ions, may have, for example, a dramatic impact on numerous vital phosphate-involving enzymes, either as inhibitors or as activators.

The actions of certain growth factors, such as epidermal growth factor, insulin, and platelet-derived growth factor, involve an enzymatic phosphorylation of the receptor. Vanadate, as a phosphate analog or substitute, may inhibit the enzymatic dephosphorylation of the receptor and thus mimic many of the phosphate-dependent actions of such growth factors.

Vanadate shares mitogenic activity with epidermal growth factor, platelet-derived growth factor, vasopressin, prostaglandin F_2alpha, bradykinin, phorbol esters, lipopolysaccharide, and lectins. Both vanadate and these agents are capable of activating chloride-sensitive sodium ion/hydrogen ion exchange and calcium ion fluxes across cell membranes and can thereby alter ionic composition of cells. Vanadate ions enter cells where they are reduced to vanadyl ions and bound to endogenous ligands. The bulk of all vanadium in the body is intracellular and is in the form of vanadyl. The function of vanadyl ions is even less clear than that of vanadate ions.

Vanadium is needed for optimal growth of fibroblasts in tissue culture. Vanadium has insulin-like properties at the level of the cell and whole animal. Vanadium also stimulates the formation of new blood vessels.

A preferred feature f the present invention is to provide a method, substantially free of undesirable side effects, that uses vanadates alone or in combination with other compounds to improve the appearance of aged or chronically sun-damaged human skin.

Another important preferred feature of the present invention is to provide a method, substantially free of undesirable side effects, that uses such vanadates to aid in healing of skin and corneal wounds and lesions as well as healing of internal conditions and organs such as kidney, heart and brain.

An important aspect of the present invention comprises a method of alleviating skin wrinkles and other effects of aging on the skin using vanadium formulations or compositions. The method includes repeatedly applying vanadium compositions in a selected concentration over a period of time.

Another feature of the present invention comprises a method of promoting wound healing following skin injury or trauma and accelerating regeneration or healing of dermal skin injuries. Vanadium compositions and treatments of this invention will also promote healing of corneal abrasions and increase tensile strength of skin and corneal incisions. The methods of the invention usually include repeated application of specifically formulated vanadium compositions over a period of time although single applications for extended contacts, such as provided by various transdermal application techniques, are also within the scope of this invention.

Still an additional feature of the present invention comprises a m th d for healing of injuries f renal epithelial cells due to disease or aging and f promoting

healing of ischemic lesions of the heart and brain. The method includes oral and par nteral routes of delivery.

The following prescriptions are presented as elucidating and are not meant to represent limitations of the invention unless otherwise specifically indicated herein.

Water-soluble, vehicle-soluble and compounds capable of forming fine suspensions in water or in a vehicle are suitable for preparing vanadium compositions useful in this invention. Vanadium compounds with valence numbers of 3, 4 and 5 are usable, but 4 and 5 are preferred, and those with valence 5 are especially preferred. In general, regardless of the composition used initially, the compound will be converted in solution to forms specific for a given pH. So that skin irritation will be avoided extreme ranges of pH such as 1-4 and 9-14 should not be used. Additionally, the vanadium compound should dissolve in water or vehicle, or form fine suspensions to attain a minimal concentration of at least 4.5×10^{-8} M to 1×10^{-7} M. The higher concentration limit is suggested when a vanadium compound is used alone. When used with other complementary adjuvants as disclosed elsewhere herein, much lower concentration limits, e.g. as low as 2×10^{-8} M, can be used. An upper concentration of 5×10^{-6} M at the tissue surface is preferred, 5×10^{-8}M to 5×10^{-7} M being most preferred, and 10^{-7}M especially preferred. Although vanadates have been demonstrated to be nontoxic in concentrations of 3% to 30%, which is about 0.25 M to 2.5 M, expressed as vanadate (U.S. Patent 4,490,354), upper limits of 5×10^{-4}M are preferred to avoid skin irritations, but the 0.25 M to 2.5 M concentrations and above can sometimes be used for non-antiperspirant uses when accelerated results are desired and especially when in combination with adjuvants and modes such as

transdermal techniques. In most instances, concentrations of 0.25 M to 2.5 M are much too high to be effectively utilizable by the tissue to be treated unless metered out in some manner. It should be noted that the especially preferred concentration used herein of 1 X 10^{-7} M is 2.5 to 25 million times less than concentrations specified for antiperspirant use by U.S. Patent 4,490,354 which is incorporated by reference herein.

Examples of suitable vanadium compounds useful in the practice of the present invention include:

1. Pentavalent compounds: Sodium metavanadate ($NaVO_3$), sodium orthovanadate (Na_3VO_4), sodium pyrovanadate ($Na_4V_2O_7$), corresponding salts with potassium (KVO_3), ammonium (NH_4VO_3), calcium ($Ca_3(VO_4)_2$), iron ($Fe(VO_3)_3$), and corresponding salts of vanadates with magnesium, zinc, aluminum, and the like. Vanadium pentoxide (V_2O_5), vanadium oxytrichloride ($VOCl_3$), vanadium oxytribromide ($VOBr_3$) and the like. Polymers such as a dimer ($H_2V_2O_7$), a trimer (V_3O_9), a decamer ($HV_{10}O_{28}$), and the like.

2. Tetravalent compounds: Vanadyl sulfate ($VOSO_4$), and corresponding compounds with acetate, etc. Vanadium (IV) oxyhalides such as vanadium oxychloride ($VOCl_2$), vanadium oxydibromide ($VOBr_2$), vanadium oxydifluoride (VOF_2). Vanadium (IV) halides such as vanadium tetrachloride (VCl_4), vanadium tetrabromide (VBr_4) and vanadium tetrafluoride (VF_4) and the like. Vanadium dioxide (VO_2) and vanadium tetraoxide (V_2O_4).

3. Trivalent vanadium compounds: halides of vanadium (III) such as vanadium trichloride (VCl_3), vanadium tribromid (VBr_3), vanadium trifluoride (VF_3), vanadium triiodid (VI_3). Oxyhalides of vanadium (III)

such as vanadium oxyclhloride (VOCl), vanadium oxybromide (VOBr), and the lik .

4. Chelates, clathrates or other complexes of vanadium, including those for slow release. Some specific vanadium complexes which are useful include those with amino acids, proteins, epidermal growth factor, other growth factors, nucleic acids, phosphates, phospholipids, fatty acids, prostaglandins, citrates, ascorbate, fruit acids, retinoids, retinol, retinal, tris, edatate, glycols, catechols, glutathione, and the like.

5. Organic vanadium compounds such as salts of organic acids or vanadium contained in tunicates (sea squirts), some mushroom species and plants, and other organic sources. Specific examples of vanadium organo-metallic compounds include vanadyl salts of organic acids such as: vanadyl linoleate, oleate, palmitate, phenolate, resinate and stearate.

Typically, but not so limited, concentrations of about 5×10^{-8} M to about 1×10^{-7} M of vanadium-containing compounds or complexes based on concentration of vanadate may be applied in the practice of the present invention. For skin treatment, one effective regimen for applying the vanadium-containing composition is twice daily at least until wrinkles are alleviated and once a day thereafter. Sustained-release preparations can be applied less frequently, such as every other or every third day. Highly concentrated preparations can also be applied only once a day or less.

Vanadium-containing compounds will act synergistically with epidermal growth factors and/or retinoids to facilitate improvement of skin structure and appearance, and these combinati n comp unds, esp cially

formulated to minimize retinoid irritation and toxicity, should be applied once or twice daily until wrinkles and related cosmetic conditions are alleviated and once daily thereafter. Therefore, vanadium-containing compositions containing optimal functionality with minimum toxicity are achievable and preferred for cosmetic applications, and much higher vanadium concentrations can be used to achieve maximum functionality without toxicity. For inter vivo applications, toxicity is not a factor, and much higher concentrations of vanadium and other active ingredients necessary to achieve significant improvement according to this invention. Again, sustained-release preparations may be applied less frequently, such as every other or every third day.

Compounds containing alpha-hydroxy acids, such as those derived from fruits (apples, grapes, oranges, lemons, etc.), also known as fruit acids, tartaric acid being a specific example, are particularly useful for cosmetic purposes in admixture with vanadium compounds alone and in admixture with vanadium compounds and other adjuvants such as retinoids. The concentrated acids are most effective. Retin-A and alpha-hydroxy acids have an irritating skin abrading effect. That effect enables the vanadium-containing compositions to penetrate more effectively below the outer layer and interact with mechanisms in the skin and as new collagen is formed or replaced.

In preparing a vanadium composition suitable for processes of the invention, a carrier is typically used, such a carrier may include treatment compositions typically vended by the skin care industry. Treatment compositions well-known in the industry include, for example, MULTIMOISTURE (Neiman Marcus), CLINIQUE, NIVEA, LUBRIDERM SKIN CARE, EUCERIN, and FORMULA No. 499 MO-DAGE

MOISTURIZING EMOLLIENT LOTION (AARP Pharmacy Dervice). The carrier is preferably a standard and cosmetically desirable one. Additionally, the preparation created preferably should physically maintain the vanadium on the skin for much of the time between applications at intervals as detailed above.

In accordance with the present invention, the water-soluble vanadium salts are incorporated into compositions comprising the salt and a cosmetically or pharmaceutically acceptable compatible carrier. By "cosmetically or pharmaceutically acceptable compatible carrier" is meant any material, or combination of materials, which aids in the delivery of the vanadium salt to the tissue area to be treated, is non-toxic or cosmetically non-offensive from a patient or consumer standpoint, and does not materially adversely interfere with the healing or wrinkle alleviating activity of the vanadium salt. Numerous compounds and systems meet these criteria and the use of same is well known to the art. Exemplary carriers, particularly for topical applications, are: water, humectants such as glycerol, lower alkyl alcohols such as ethanol and isopropanol, natural waxes, such as beeswax, spermaceti and cresin, plant extracts such as those of Aloe vera, hydrocarbon waxes, hydrocarbon oils such as mineral oil, C_{12}-C_{22} straight chain alcohols and fatty acids, volatile silicones, suspending agents such as talc and starch, halogenated hydrocarbons such as trichloromonofluoromethane, dichloro-difluoromethane and dichloro-tetrafluoroethane, and the like. Obviously, compatible combinations of these carriers may also be used.

Of course, other additives may be incorporated into t pically applied compositi ns, such as, for exampl , fragrances, dispersing agents, binding agents, color-

masking agents and the lik , depending upon the particular type of product into which the vanadium salt is to be incorporated.

Vanadium salts applied to the skin have very low toxicity and skin irritation levels, even at concentrations much higher than necessary to achieve significant improvement according to this invention (see United States Patent No. 4,490,354). Also, it is generally believed that vanadium penetrates the tissues poorly. Thus, the results in the examples using topical applications of any vanadium concentration are particularly surprising because the changes are believed to occur in subcutaneous tissues.

The invention is further illustrated by the following Examples.

EXAMPLE 1

REJUVENATING EFFECT OF VANADIUM ON SUN-DAMAGED SKIN OF FACE AND NECK: A 144 DAY STUDY

Further details are explained below with the help of the examples illustrated in the accompanying figures. Figure 1A is a frontal view of a human subject before treatment. Figure 1B is a frontal view of the subject after 40 days of application of a vanadate composition (described below) twice daily to the right side of his face and application of the vehicle alone (placebo) used in the vanadate composition twice daily to the left side. Both sides of the neck were treated twice daily with the same vanadate composition. Figure 1C is a frontal view of the subject after 48 days of twice daily application of the vanadate composition to the right side and placebo application to the 1 ft side. B th sides of the neck were treated twice daily with the vanadate c mposition. Figure

1D is frontal view of the subject after 58 days of twice daily application of the vanadate comp sition to the right side and placebo application to the left side. Both sides of the neck were treated twice daily with the vanadate composition.

Figure 1E is a left perspective view of the subject after 48 days of twice daily treatment with placebo. Both sides of the neck were treated twice daily with the vanadate composition. Figure 1F is a right perspective view of the subject after 48 days of twice daily treatment with a vanadate composition. Both sides of the neck were treated twice daily with the vanadate composition. Figure 1G is a left perspective view of the subject after 58 days of twice daily treatment with placebo. Both sides of the neck were treated twice daily with the vanadate composition. Figure 1H is a right perspective view of the subject after 58 days of twice daily treatment with a vanadate composition. Both sides of the neck were treated twice daily with the vanadate composition. Figure 1I is a left perspective view of the subject after 86 days of twice daily treatment with a vanadate composition. Both sides of the neck were treated twice daily with a vanadate composition. Figure 1J is a right perspective view of the subject after 144 days of twice daily treatment with a vanadate composition. Both sides of the neck were treated twice daily with the vanadate composition.

A dilute solution of sodium vanadate ($NaVO_3$) (5×10^{-8} M) which contained 2.55 nanograms (ng) of vanadium per milliliter, in 95% glycerol, U.S.P. and 5% water was prepared.

A subject (subject 1) was selected, photographed (see Fig. 1A), and beginning on July 11, 1987, vanadate solution is 95% glycerol, U.S.P. and 5% water was applied

topically to the right side of the subject's face and both sides of the subject's neck twice daily for 58 days. The left side of the subject's face was treated with a mixture of 95% glycerol, U.S.P. and 5% water (placebo).

Frontal facial photographs were taken after treatment twice daily for 40 days (Fig. 1B), 48 days (Fig. 1C) and 58 days (Fig 1D). As shown in Figures 1B-1D, the vanadium-based treatment of the right side caused significant lessening of facial lines and wrinkles. Wrinkles and sagging appearance of the neck that are apparent in the pre-treatment condition (Fig. 1A) largely disappeared after 40 days of treatment (Fig. 1C), and this improvement continued to be present at 48 and 58 days after the beginning of treatment (Fig. 1C, 1D). Bags under the eyes were also diminished on the right side.

Figures 1F-1H show side facial photographs of the treated subject. Placebo application to the left side of the face for 48 days (Fig. 1E) and for 58 days (Fig. 1G) resulted in no change in appearance of the left side. Treatment of the right side with the vanadium preparation for 48 days (Fig. 1F) and for 58 days (Fig. 1H), show the rejuvenating effects of vanadium treatment. Facial wrinkles (e.g., crow's feet) and deep facial lines are diminished on the right (vanadium-treated) side, and skin on the right side appears fuller and firmer.

After 58 days, the vanadate preparation was applied twice daily to both sides of the face and neck: the photographs in Figs. 1I and 1J were taken 86 days later. Fig. 1I shows the effect of a total of 86 days of twice-daily vanadate application to the left side of the face. Fig. 1J shows the cumulative effect of a total of 144 days f twice daily treatment of th right side f the face and n ck with the vanadium preparation. At this time,

wrinkles appeared more similar on both sides of the face, i.e., the left side improved and als began to show fewer wrinkles and deep facial lines.

Not readily appreciated by viewing the photographs are the following effects, which were noted by the subject:

1. Skin treated with vanadium felt softer, was less puffy, was less red (erythematous), and was more elastic;

2. Less redness (erythema) was present around the pre-cancerous lesions, actinic keratoses.

3. Bags under the eyes were decreased. This effect was also apparent on the frontal photographs.

Thirty-Six randomly selected adults were shown the facial photographs in Figures 1A-1J (without the numerical insets) and were asked questions regarding the appearance of the skin. The respondents were not aware of the treatment regimen. The questions, number of responses and percentage of responses to each question are shown below in Tables 1-I through 2-III.

TABLE 1-I

FOR FIGURE 1A-1D

QUESTION 1. Do you think that one side of the face presents a younger appearance in the photographs labeled 1A through 1D? If so, which side appears younger

ANSWERS.

	FIGURE: 1A		1B		1C		1D	
	#[1]	%[2]	#[1]	%[2]	#[1]	%[2]	#[1]	%[2]
Both sides about the same	21	58.3	7	19.4	2	5.6	3	8.3
Left side slightly younger than right	0	0	0	0	0	0	0	0
Right side slightly younger than left	10	27.8	9	25.0	3	8.3	7	19.4
Left side much younger than right	0	0	0	0	0	0	0	0
Right side much younger than left	4	11.1	14	38.9	5	13.0	13	63.9
Left side very much younger than right	0	0	0	0	0	0	0	0
Right side very much younger than left	1	2.8	6	16.7	26	72.2	3	8.3
Totals	36	100.0	36	100.0	36	100.0	36	100.0

[1]number answering

[2]percent of total respondents

As indicated by the above results, the impartial respondents perceived a definite rejuvenating effect of vanadate treatment on the right side of the subject's face.

TABLE 1-II
FOR FIGURES 1A-1D

QUESTION 2. Which figure shows most wrinkles on the right side of the face?

ANSWERS.

	Figure:					
	1A	1B	1C	1D	Neither	Totals
#	30	0	0	2	4	36
%	83.3	0	0	5.6	11.1	100

TABLE 1-III
FOR FIGURES 1A-1D

QUESTION 3. Which figure shows most wrinkles on the left side of the face?

ANSWERS.

	Figure:					
	1A	1B	1C	1D	Neither	Totals
#	1	3	3	6	23	36
%	2.8	8.3	8.3	16.7	63.9	100

TABLE 1-IV
FOR FIGURES 1A-1D

QUESTION 4. Which figure shows most wrinkles on the neck?

ANSWERS.

	Figure:					
	1A	1B	1C	1D	Neither	Totals
#	36	0	0	0	0	36
%	100	0	0	0	0	100

*I902*The responses to questions 2-4 further support the trend of responses to Question 1 in establishing the affectiveness of vanadate treatment.

TABLE 1-V
FOR FIGURES 1E-1J

QUESTION 5. Which figure shows the most wrinkles on the whole face?

ANSWERS.

	Figure:							
	1E	1F	1G	1H	1I	1J	Neither	Totals
#	7	0	29	0	0	0	0	36
%	19.4	0	80.6	0	0	0	0	100

TABLE 1-VI
FOR FIGURES 1E-1J

QUESTION 6. Which pair of pictures shows the greatest difference in the amount of total wrinkles?

ANSWERS.

Figures:

	1E & 1F	1G & 1H	1I & 1J	Wrinkles about the same in each pair of Figures	Totals
#	1	35	0	0	36
%	2.8	97.2	0	0	100

TABLE 1-VII
FOR FIGURES 1E-1J

QUESTION 7. Which pair of pictures shows the least differences in the amount of wrinkles?

ANSWERS.

Figures:

	1E & 1F	1G & 1H	1I & 1J	Wrinkles about the same in each pair of Figures	Totals
#	5	0	31	0	36
%	13.9	0	86.4	0	100

The response to these additional questions are further consistent with a perceived vanadate-induced rejuvenation effect on the skin.

EXAMPLE 2
REJUVENATING EFFECT OF VANADIUM ON THE FACE: A 16 DAY STUDY

Further details are explained below with the help of the examples illustrated in the accompanying Figures. Figure 2A is a frontal view of the 47-year old subject before treatment. Figure 2B is a frontal view of the subject after 16 days of application of a vanadate composition (described below) twice daily to the left side of the face and application of the vehicle used in the same vanadate composition, herein called 'placebo,' twice daily to the right side of the face.

A dilute solution of sodium vanadate (1×10^{-7} M ($NaVO_3$), which contained 5.1 nanograms of vanadium per milliliter, in 95% glycerol, U.S.P. and 5% water was used. Beginning on August 20, 1988, the vanadate solution was applied topically to the left side of a second subject's face twice daily. The right side was treated twice daily with a mixture of 95% glycerol, U.S.P. and 5% water, the vehicle in which the vanadium was dissolved, hereinafter called 'placebo.'

Frontal facial photographs were taken before treatment (Fig. 2A) and after 16 days of treatment (Fig. 2B). After 16 days of treatment, the left side of the subject's face had fewer wrinkles lateral to the eye (crow's feet), decreased darkness and bags under the eye and a decrease in the dark, hollow area in the middle of the cheek as compared to the left side before treatment and to the right side before and after placebo application.

Thirty randomly selected adults shown the facial photographs in Figures 2A and 2B and asked questions regarding the appearance of the skin. The respondents were not aware of the treatment regimen. The questions, number and type of responses to each question is shown below.

TABLE 2-I
FOR FIGURES 2A-2B

QUESTION 1. In the two figures, do you think that one side of the face appears younger? If so, what side appears younger?

	Figure 2A		Figure 2B	
Responses:	#	%	#	%
Both sides about the same	20	66.7	6	20.0
Left side younger than right	6	20.0	22	73.3
Right side younger than left	4	13.3	2	6.7
Totals:	30	100.0	30	100.0

TABLE 2-II
FOR FIGURES 2A-2B

QUESTION 2. Which figure shows a greater amount of crow's feet around the eye?

	Figure 2A		Figure 2B	
Responses:	#	%	#	%
Left side	15	50.0	8	26.7
Right side	15	50.0	22	73.3
Totals:	30	100.0	30	100.0

TABLE 2-III
FOR FIGURES 2A-2B

QUESTION 3. Which figure shows greater bagginess under the eye?

	Figure 2A		Figure 2B	
Responses:	#	%	#	%
Left side	14	46.7	6	20.0
Right side	16	53.3	24	80.0
Totals:	30	100.0	30	100.0

The responses to these questions support a perception of rejuvenating effects by vanadate treatment on the left side of the subject's face.

EXAMPLE 3
HEALING EFFECT OF VANADIUM ON
FROZEN ACTINIC KERATOSES

Figure 3A is a dorsal view of a subject's hands 48 hours after actinic keratoses were frozen with liquid nitrogen. Twenty-four hours after liquid nitrogen application, the hands were treated on a twice daily basis. The right hand was treated with vanadate, and the left hand was treated in the same manner but with the vehicle used for the vanadate mixture alone (placebo). Figure 3B is a dorsal view of the subject's hands after four days of treatment with vanadate (right hand) and with placebo (left hand). Figure 3C is a dorsal view of the subject's hands after fourteen days of treatment with vanadate (right hand) and with placebo (left hand).

A dilute solution of sodium vanadate (1×10^{-7} M $NaVO_3$), containing 5.1 nanograms of vanadium per milliliter, in 95% glycerol, U.S.P. and 5% water was used.

On August 31, 1988, actinic keratoses of about 5 mm diameter on both hands were sprayed with liquid nitrogen. The procedure resulted in local freezing and in death of cells of the sprayed lesions and surrounding inflammation. Scab formation occurred and was followed by regeneration of healthy skin when the scabs fell off. A vanadium composition in a concentration of 1×10^{-7} M was applied twice daily to the right hand, beginning 24 hours after liquid nitrogen had been sprayed on actinic keratoses. Placebo was applied in a similar fashion to the left hand. Figure 3A is a photograph taken 24 hours after vanadium treatment and placebo application began. Lesions of the placebo-treated left hand (arrow) showed more swelling and redness than the lesions on the vanadium-treated right hand. Scabs on the vanadium-treated right hand appeared darker and less raised and had less surrounding erythema than scabs on the placebo-treated left hand, indicating that better healing occurred.

Figure 3B is a photograph taken four days after vanadium and placebo application began. Erythema of lesions of the placebo-treated left hand persisted (arrow). Lesions of the vanadium treated right hand had less surrounding erythema, darker coloration and were less raised (arrow).

Figure 3C is a photograph taken fourteen days after vanadium and placebo application began. The largest scab on the vanadium-treated side (arrow) had fallen off and healed normal skin appeared beneath the lesion. Healing of lesions of the placebo-treated left hand was not as rapid.

* * * * * * * * * * * *

As a concluding disclosure of the invention described herein, it is to be noted that not only may retinoic acid be irritating and even toxic at relatively low dosages, but it is only effective for small wrinkles. The vanadium compositions of the present invention are not only nontoxic, even at relatively higher levels, but are effective for reducing bags under the eyes and deep wrinkles. Therefore, as suggested above, it forms a feature of this invention that there is a likely synergism between retinoids, e.g., retinoic acid and vanadium-containing compositions to combine the best features of both to achieve, either cosmetic improvement (particularly of the face), improved tissue growth and improved tissue healing, without toxicity. These are regarded as very important features of this invention.

In the same vein, use of vanadium compounds in sensitive areas such as corneas for alleviating damages, corneal abrasion, for example, will be useful either alone or in combination with materials such as epidermal growth factors. These are usually proteins that can be produced by recombinant microorganisms. Other specific members of the growth factor family include: fibroblast, platelet-derived, insulin-like, transforming and nerve growth factors. Vanadium compounds in combination with any of these factors in quantities chosen to have a measurable beneficial effect, as compared to identically treated tissue without vanadium, will result in new compositions of matter of special importance and utility in these areas.

Although it is recognized that the prior art has used rather high concentrations of 0.25 M to 2.5 M vanadate as an perspiration inhibitor for underarm use and that humans

have bathed in sea water containing maximal concentration of about 4 x 10^{-8} M vanadate, it is not been previously taught that vanadate compounds of any concentration between that of sea water and antiperspirant have any effect on any tissue. Furthermore, at concentrations in the general range of 0.25 M to 2.5 M vanadate, only antiperspirant effects were sought to be achieved and only in the specific locality of underarms. Underarm tissue has been treated for reducing perspiration by using high quantities of vanadate to interact with sweat glands and retain a salt plug therein to retard perspiration. Even if this may have inherently caused tissue change, it was not sought or observed by the art. If tissue changes are only found in other areas, particularly visible ones which have not been treated with sufficient amounts of vanadium to cause antiperspirant effects, then it is likely such tissue may have been indirectly contacted with therapeutic levels of vanadium. A tissue which does not have the ability to absorb or utilize any more than the amounts of vanadate set forth in the general use range of the present aqueous-based compositions containing 4.5 x 10^{-8} M to 5 x 10^{-7} M vanadate or metal-organic equivalent may show cosmetic or therapeutic improvement because only the therapeutic amounts of vanadium-containing described herein are functional. Any amounts over this are considered unnecessary with respect to the cosmetic, healing and other therapeutic effects, all of which are the achievements of this invention. Such use of excess vanadium would not be effective as a stratagem to avoid the doctrine of equivalents to be afforded claims for the generally lower concentration range of the present invention.

As a very general principle, the benefits of the present invention are deemed established or achieved when exposure of tissue to a certain regimen of treatment by

periodic contact with vanadium-containing compositions, as disclosed herein, shows measurable improvement in at least one of cosmetic appearance, healing or accelerated tissue growth, as compared to identical treatment of identical tissue with compositions identical except that they are non-vanadium-containing.

The vanadium-containing compounds of this invention may be formulated and used directly in an aqueous based composition. But, it is preferred such compositions be formulated appropriately for specific conditions in accordance with the established art and practices of pharmacy. Methods of administration, although preferably direct, may include indirect enteral or parenteral methods. Such indirect methods should result in contact of tissue being treated with the preferred therapeutic levels of vanadium-containing compounds described herein.

As has been disclosed above, it is clear that the general scope of the invention contemplates the use of the disclosed vanadium compounds in admixture with a wide variety of other substances to receive the benefit of the combination. In addition to retinoids, such as Retin-A and growth enhancing compounds, those compositions, such as aloe, now or in the future in the marketplace for skin enhancing purposes are ideally suited and adapted for compounding with the vanadium compositions of this invention. Moreover, since the vanadium compounds of the invention appear to have a beneficial effect on collagen, they may be beneficially used alone or in combination with conventional materials available for use in treating joints, etc. for gouty or arthritic conditions. In such applications, and in certain others as will be apparent to those skilled in the art, the vanadium in admixture with compounds which enhance skin penetration forms particularly useful compositions.

Numer us changes may be mad in the particular vanadium-containing compounds and compositions described herein, the concentrations thereof, or in the steps of the sequence of steps of the method described herein without departing from the concept and scope of the invention as defined in the following claims.

CLAIMS:

1. A process for therapeutically improving the cosmetic appearance, growth or healing characteristics of mammalian tissue, comprising contacting mammalian tissue with a therapeutically effective amount of a vanadium-containing composition comprising a vanadium-containing compound, whereby the amount of vanadium present as measured by vanadate ion is between about 4.5×10^{-8} M and less than 0.25 M, until the improvement exceeds that experienced under identical conditions except that none or a lesser quantity of vanadium-containing compound is so applied.

2. The process of claim 1 wherein said vanadium-containing compound is vanadate ion or a similar vanadium (V) compound.

3. The process of claim 1 wherein said compound is vanadyl ion or a similar vanadium (IV) compound.

4. The process of claim 1 wherein said compound is a vanadium-containing organic compound.

5. The process of claim 1 wherein said vanadium-containing compound at concentrations of 4.5×10^{-8} M to 5×10^{-7} M is contacted with said tissue for a time of at least 30 to 120 minutes for one to four times per day until at least noticeable growth or healing or cosmetic improvement is obtained.

6. The process of claim 1 wherein said tissue is human or animal and the concentrati ns f vanadium-containing compound is between at 5 x 10^{-8} M and 5 x 10^{-6} M.

7. The process of claim 6 wherein said tissue is externally located on a human or animal body.

8. The process of claim 6 wherein said tissue is internally located within a human or animal body.

9. The process of claim 1 wherein said tissue is in an in vitro environment extrinsic to a human or animal.

10. The process of claim 1 where said tissue is in vivo to a human or animal.

11. A method of treating mammalian tissue comprising:

a) preparing a composition comprising a vanadium-containing compound and a carrier where said composition comprises at least 4.5 x 10^{-8} M vanadium in an aqueous soluble form;

b) selecting mammalian tissue in need of treatment for improved cosmetic appearance, improved healing, or a faster rate of tissue cell growth;

c) applying the composition to tissue in need of treatment at least until tissue is healed, rejuvenated or cosmetically improv d to visual inspecti n.

12. A method for preventing and alleviating wrinkles in skin tissue comprising:

a) preparing a composition comprising a vanadium-containing compound and a carrier;

b) selecting wrinkled or potentially wrinkled skin areas;

c) applying the composition repeatedly to said selected skin areas.

13. The method of claim 11 or 12, wherein the vanadium of the vanadium-containing compound has a valence number of 3, 4, or 5.

14. The method of claim 11 or 12, wherein the carrier has a pH between about 6 and about 8.

15. The method of claim 11 or 12, wherein the vanadium-containing compound is dissolved in the carrier to produce a solution of at least 5×10^{-8} M.

16. The method of claims 11 or 12, wherein the vanadium-containing compound is: sodium metavanadate ($NaVO_3$), sodium orthovanadate (Na_3VO_4), sodium pyrovanadate ($Na_4V_2O_7$), corresponding salts with potassium (KVO_3), ammonium metavanadate (NH_4VO_3), calcium orthovanadate ($Ca_3(VO_4)_2$), ferric metavanadate ($Fe(VO_3)_3$), corresponding vanadate salt with magnesium, zinc, aluminum, vanadium pent xid (V_2O_5), vanadium oxytrichloride ($VOCl_3$), or vanadium oxytribromide ($VOBr_3$).

17. The method of claims 11 or 12, wherein the vanadium-containing comp und is a tetravalent vanadium compound: selected from the group consisting of vanadyl sulfate ($VOSO_4$), vanadium oxydichloride ($VOCl_2$), vanadium oxydibromide ($VOBr_2$), vanadium oxydifluoride (VOF_2), vanadium tetrachloride (VCl_4), vanadium tetrabromide (VBr_4), vanadium tetrafluoride (VF_4), vanadium dioxide (VO_2) and vanadium tetraoxide (V_2O_4).

18. The method of claims 11 or 12, wherein the vanadium-containing compound is a trivalent vanadium compound: selected from the group consisting of vanadium trichloride (VCl_3), vanadium tribromide (VBr_3), vanadium trifluoride (VF_3), vanadium triiodide (VI_3), vanadium oxychloride (VOCl), vanadium oxybromide (VOBr), vanadium oxyfluoride and vanadium oxyiodide.

19. The method of claims 11 or 12, wherein the vanadium-containing compound is a vanadium salt of an organic acid, vanadium compound contained in tunicates, certain mushroom species or plants.

20. The method of claim 11 or 12, wherein the vanadium-containing compound is a dimer (HV_2O_7), a trimer V_3O_9, a decamer ($HV_{10}O_{28}$) or other polymeric form.

21. The method of claim 11 or 12, wherein the applying step is defined further as being twice daily.

22. The method of Claim 11 or 12, wherein the preparing step is defined further as preparing a composition comprising a vanadium-containing compound in a concentration between about 4.5×10^{-8} M and about 0.25 M.

23. The method of claim 11 or 12, wherein the carrier is defined further as aiding in the retention of the vanadium-containing compound on the skin.

24. The method of claim 11 or 12, wherein the vanadium-containing compound is a chelate with amino acids, proteins, epidermal growth factor, other mammalian growth factors, nucleic acids, phosphates, phospholipids, citrate, ascorbate, fruit acids, retinoids, retinol, retinal, tris, edatate, glycols, catechols, glutathione, alpha-hydroxy acids, prostaglandins, or fatty acids.

25. The method of claim 11 or 12, wherein the composition prepared in the preparing step comprises an epidermal growth factor.

26. The method of claim 11 or 12, wherein the preparing step is defined further as preparing a composition comprising a vanadium-containing compound at a concentration of about 1×10^{-7} M to 5×10^{-7} M.

27. The method of claim 11 or 12, wherein the composition produced in the preparing step comprises a retinoid.

28. The method of claim 12, wher in the applying step is defined further as applying said composition t facial and throat skin.

29. The of claim 28 where said composition comprises a cosmetically altering amount of an alpha-hydroxy acid.

30. The method of claim 11 or 12, wherein the applying step is defined further as alternating applications of the vanadium-containing compound and a retinoid.

31. The method of claim 11 or 12, wherein the applying step is defined further as alternating applications of the vanadium-containing compound and an epidermal growth factor.

32. The method of claim 11 or 12, wherein the preparing step involves vanadium having a valence charge of +1 or +2.

33. The method of claim 11 or 12, wherein the preparing step comprises dissolving a vanadium-containing compound in a concentration between about 1×10^{-7} M and about 5×10^{-6} M.

34. A composition of matter comprising a vanadium-containing compound wherein the amount of vanadium present as measured by vanadate ion is about 4.5×10^{-8} M up to less than 0.25 M.

35. Th composition of claim 34 wherein the vanadium present is a component of the vanadium compounds of claim 16 or a metallo-organic vanadium compound.

36. The composition of claim 34 wherein the vanadium is present in at least one of the compounds of claims 24.

37. A composition of matter comprising a vanadium-containing compound and a pharmaceutically or cosmetically acceptable carrier, wherein the amount of vanadium present as measured by vanadate ion is about 4.5×10^{-8} M up to less than 0.25 M.

38. The composition of claim 37 wherein the vanadium present is a component of the vanadium compounds of claim 16 or a metallo-organic vanadium compound.

39. The composition of claim 37 wherein the vanadium is present in at least one of the compounds of claims 24.

40. The composition of claim 37 wherein the pharmaceutically or cosmetically acceptable carrier comprises one or more of water, humectant, lower alkyl alcohol, natural wax, spermaceti, cresin, hydrocarbon wax, hydrocarbon oil, plant extract, C_{12}-C_{22} straight chain alcohol, straight chaim fatty acid, and volatile silicone.

41. Th composition of claim 37 defined further as comprising a fruit acid.

42. Th composition of claim 37 defined further as comprising one or more of retinoid, mammalian growth factor.

43. The composition of claim 37 defined further as comprising a skin care composition.

44. The composition of claim 33 wherin the skin care composition is defined further as being commercially available.

FIG.1A

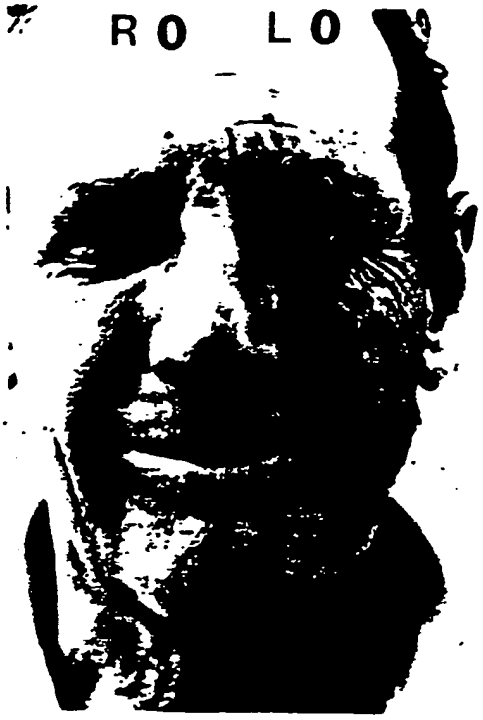

FIG.1B

FIG.1C

FIG.1D

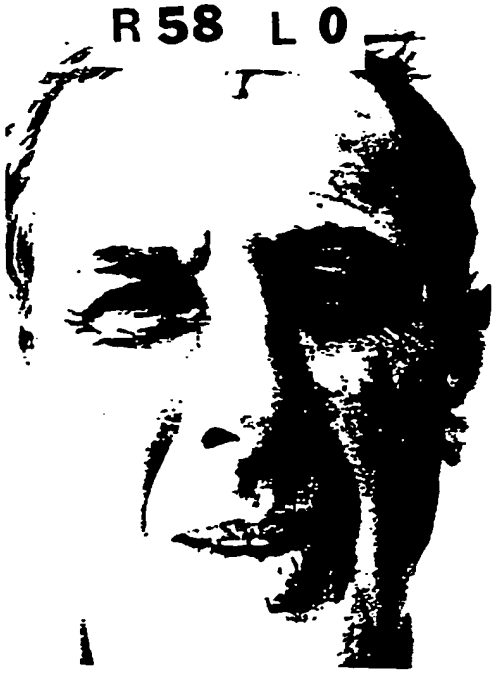

FIG.1E

FIG.1F

FIG.1G

FIG.1H

FIG.1J

FIG.1I

FIG.2A

FIG.2B

FIG.3A

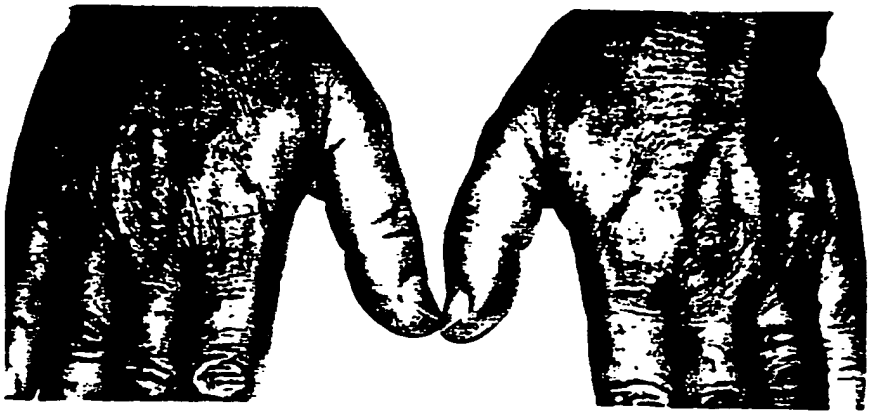

FIG.3B

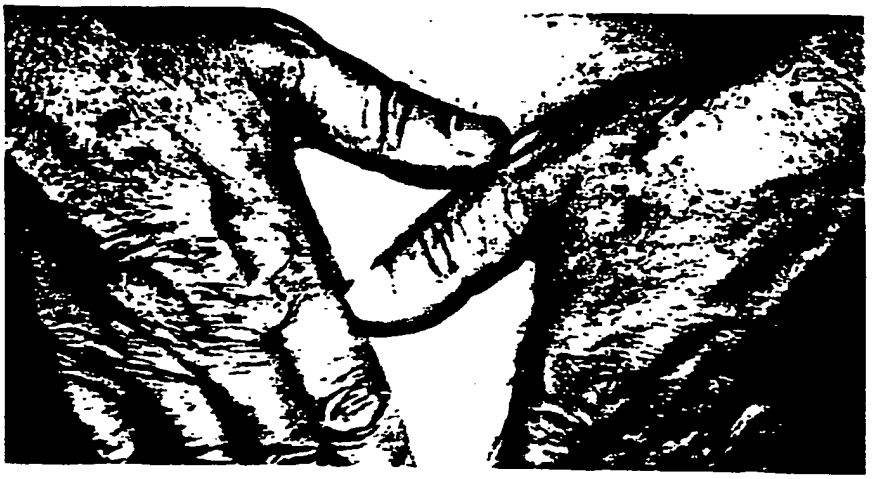

FIG.3C

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 90/02175

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[5]: A 61 K 7/48, 33/24, 31/28

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC[5]	A 61 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X	DE, A, 3327840 (BLENDAX) 20 September 1984 see claims 1,3-6; example 8; column 2, lines 17-20; column 3, lines 30-37, 42-63	1-7,10-14, 16,22-24,27-30,34-44
X	GB, A, 1270410 (ALLOR CORPORATION) 12 April 1972 see the whole document	1,4,7,10-12, 22-24,28, 34-40,43-44
X	EP, A, 0152366 (LE RIBAULT et al.) 21 August 1985 see the whole document	34-44
X	EP, A, 0274404 (KAPLAN) 13 July 1988 see claims; examples	34-44

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* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
11th September 1990	11. 10. 90
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	R.J. Eernisse

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, 11 with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X	DE, A, 2923334 (KÖPF et al.) 11 December 1980 see the whole document --	34-44
X	FR, A, 2429021 (BERES) 18 January 1980 see the whole document --	34-44
X	WO, A, 88/03805 (CHEMEX PHARMACEUTICALS INC.) 2 June 1988 see page 29, paragraph 6 - page 30, paragraph H; page 34, paragraph H-I; claims 7-55 --------	34-44

FURTHER INFORMATION ONTINUED FR M THE SECOND SHEET

V. [X] OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. [X] Claim numbers .....*..., because they relate to subject matter not required to be searched by this Authority, namely:

* Claims searched completely:12,28-29,34-44.
Claims searched incompletely:1-7,9-11,13-27,30-33
Claims not searched: 8
See PCT-Rule 39.1(IV); Methods for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods.

2. [ ] Claim numbers....., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claim numbers....., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. [ ] OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. [ ] As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

[ ] The additional search fees were accompanied by applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. US 9002175 SA 36448

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 05/10/90
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
DE-A- 3327840	20-09-84	CA-A- 1223816	07-07-87
		DE-A- 3475593	19-01-89
		EP-A,B 0155344	25-09-85
		JP-A- 60069012	19-04-85
		US-A- 4743442	10-05-88
GB-A- 1270410	12-04-72	None	
EP-A- 0152366	21-08-85	FR-A,B 2559063	09-08-85
		CA-A- 1255590	13-06-89
EP-A- 0274404	13-07-88	AU-B- 594851	15-03-90
		AU-A- 8316587	07-07-88
		JP-A- 63239227	05-10-88
		ZA-A- 8800041	24-06-88
DE-A- 2923334	11-12-80	JP-A,B,C55164624	22-12-80
		US-A- 4608387	26-08-86
FR-A- 2429021	18-01-80	None	
WO-A- 8803805	02-06-88	EP-A- 0290442	17-11-88